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Nerve excitability studies characterize $K_V1.1$ fast potassium channel dysfunction in patients with episodic ataxia type 1

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Episodic ataxia type 1 is a neuronal channelopathy caused by mutations in the *KCNA1* gene encoding the fast K^+ channel subunit $K_v1.1$. Episodic ataxia type 1 presents with brief episodes of cerebellar dysfunction and persistent neuromyotonia and is associated with an increased incidence of epilepsy. In myelinated peripheral nerve, $K_v1.1$ is highly expressed in the juxtaparanodal axon, where potassium channels limit the depolarizing afterpotential and the effects of depolarizing currents. Axonal excitability studies were performed on patients with genetically confirmed episodic ataxia type 1 to characterize the effects of $K_v1.1$ dysfunction on motor axons *in vivo*. The median nerve was stimulated at the wrist and compound muscle action potentials were recorded from abductor pollicis brevis. Threshold tracking techniques were used to record strength-duration time constant, threshold electrotonus, current/threshold relationship and the recovery cycle. Recordings from 20 patients from eight kindreds with different *KCNA1* point mutations were compared with those from 30 normal controls. All 20 patients had a history of episodic ataxia and 19 had neuromyotonia. All patients had similar, distinctive abnormalities: superexcitability was on average 100% higher in the patients than in controls ($P < 0.00001$) and, in threshold electrotonus, the increase in excitability due to a depolarizing current (20% of threshold) was 31% higher ($P < 0.00001$). Using these two parameters, the patients with episodic ataxia type 1 and controls could be clearly separated into two non-overlapping groups. Differences between the different *KCNA1* mutations were not statistically significant. Studies of nerve excitability can identify $K_v1.1$ dysfunction in patients with episodic ataxia type 1. The simple 15 min test may be useful in diagnosis, since it can differentiate patients with episodic ataxia type 1 from normal controls with high sensitivity and specificity.

Keywords: ataxia; channelopathy; nerve excitability; neuromyotonia; potassium channel

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Introduction

Voltage-gated ion channels play a critical role in the maintenance of neuronal resting membrane potential, transmission of the action potential and subsequent repolarization. Neurological diseases, either genetic or acquired, are increasingly attributed to dysfunction of neuronal ion channels (Hanna, 2006; Reid *et al.*, 2009; Kullmann, 2010). Clinical features of neuronal ion channel disorders vary widely, reflecting the function and distribution of the relevant channel in the central and/or peripheral nervous system. They may include epilepsy, migraine, ataxia, weakness and neuromyotonia. The study of genetic neuronal channelopathies can give important insights into the function of specific channels as the effects of channel mutations can be studied in detail. *In vitro* expression of mutant channels provides a means of measuring the effects of mutations on channel kinetics and ion permeation. This system has been the gold standard to confirm pathogenicity of newly identified mutations, but it can be insensitive to changes in ion channel assembly and trafficking. *In vivo* functional consequences also depend on the number of functional channels and on post-translational and environmental influences on channel function. Although effects of mutations on action potential generation and neurotransmitter release can be examined in neuronal cultures (Heeroma *et al.*, 2009), this approach relies on overexpression in an artificial system.

Diagnosis of genetic neuronal channelopathies is challenging because the symptoms may be paroxysmal and it may be difficult to recognize a phenotypic pattern. In contrast to muscle channelopathies, current clinical neurophysiological tools provide non-specific supportive evidence of altered neuronal excitability, but generally do not aid in predicting the channel involved or in guiding diagnostic testing (McManis *et al.*, 1986; Fournier *et al.*, 2004; Tomlinson *et al.*, 2009). However, it has been recently shown that nerve excitability studies can characterize the biophysical signature of single-channel dysfunction *in vivo*, in central as well as peripheral disorders (Kiernan *et al.*, 2005*a*, *b*; Krishnan *et al.*, 2006; Jankelowitz *et al.*, 2007*b*).

This study was designed to investigate possible *in vivo* changes in nerve excitability conferred by mutations in the *KCNA1* gene, which encodes the α-subunit of the fast potassium channel $K_v1.1$. In the CNS, $K_v1.1$ is highly expressed in the cerebellum and hippocampus. In the myelinated nerves of the PNS, $K_v1.1$ is concentrated at the juxtaparanode (Arroyo *et al.*, 1999; Vacher *et al.*, 2008) and contributes to the fast K^+ conductance that is activated within milliseconds of depolarization during an action potential. This conductance limits the depolarizing afterpotential following an action potential (Baker *et al.*, 1987; Schwarz *et al.*, 1995), thus preventing repetitive discharges during the superexcitable period while the nerve is recovering from the action potential (Burke *et al.*, 2001).

Mutations in *KCNA1* are associated with episodic ataxia type 1, which is characterized by brief episodes of cerebellar dysfunction lasting seconds to minutes and by persistent neuromyotonia (Browne *et al.*, 1994). Patients with episodic ataxia type 1 also have an increased incidence of epilepsy (Zuberi *et al.*, 1999). There are other phenotypes, including neuromyotonia only and distal weakness (Eunson *et al.*, 2000; Klein *et al.*, 2004; Chen *et al.*, 2007; Demos *et al.*, 2009). In the present study, nerve excitability recordings from a large cohort of patients with episodic ataxia type 1 demonstrated consistently abnormal features, attributable to a reduction of the fast K^+ conductance. These *in vivo* findings provide insight into the neurophysiological consequences of Kv1.1 dysfunction, and indicate that nerve excitability studies have the potential to aid in the diagnosis of neuronal channelopathies.

Materials and methods

Studies were performed on 20 patients from nine families, all of whom had clinical features consistent with episodic ataxia type 1 and had defined mutations of the gene *KCNA1* (Table 1). Ethical approval was obtained for the studies from the Research Ethics Committees at the University of Sydney, Australia; the University of Rochester (NY), USA; and the joint ethics committee for the National Hospital for Neurology and Neurosurgery and the UCL Institute of Neurology. All subjects provided written informed consent to participate. Patients with clinical or genetic features of episodic ataxia type 1 were recruited from a database held at each centre. Studies were performed either in the laboratories or, if the patient was unable to travel to a study site, in their homes using portable equipment.

Nerve excitability studies were performed according to the TrondE protocol using Qtrac software (written by H.B., © UCL Institute of Neurology, Queen Square, London, UK) (Kiernan *et al.*, 2000). Stimuli were applied to the median nerve at the wrist via non-polarizable adhesive electrodes (Red Dot; 3M), with the anode placed ~10 cm proximally, and displaced radially over muscle so that it did not lie over the median nerve. The compound muscle action potential was recorded using non-polarizable adhesive electrodes (Kendall, Tyco, UK) over the abductor pollicis brevis via an isolated preamplifier (LP511 amplifier; Grass Technologies). The reference electrode was placed over the distal interphalangeal joint of the thumb. The Qtrac program delivered stimuli using an isolated linear bipolar current stimulator (DS5 stimulator; Digitimer, UK) via a multifunction data-acquisition board (National Instruments; NI USB 6221). Excitability studies measure the stimulus intensity required to produce a constant fraction of the maximal compound muscle action potential. Use of identical DS5 stimulators ensured that the recordings in different settings, whether with portable equipment or in the clinical laboratories, were strictly comparable.

A stimulus–response curve was generated using 1 ms unconditioned stimuli. From the stimulus-response function, the target response was automatically set at the steepest point on the curve between 30% and 50% of the maximal response, and the 'threshold' for motor axons was defined as the stimulus current required to just evoke the target response. Subsequent measurements relate to this unconditioned threshold, which was updated throughout the recording. Once the threshold was established, the TrondE protocol measured nerve excitability in response to three different manipulations: (i) different stimulus durations (strength-duration properties); (ii) subthreshold conditioning stimuli (threshold electrotonus and current–threshold relationship); and (iii) supramaximal conditioning stimuli (the recovery cycle).

Strength-duration properties

The threshold was measured using test stimuli of 0.2, 0.4, 0.6, 0.8 and 1.0 ms duration to evaluate the strength-duration properties of the

Table 1 Summary of families with episodic ataxia type 1 studied with identified *KCNA1* mutations, and *in vitro* expression data where available

Family	Mutation	Number of subjects M:F[a]	Segment	*In vitro* expression			Reference
				Mutant channel current	Mutant + WT co-expression	Other	
1[b]	F414S	1:1 (1:0)	C-terminus	No current	Mutant exerts dominant negative effect over wild type	Positive shift in voltage dependence of activation	Graves *et al.*, 2010
2[b]	F414S	0:1	C-terminus				
3	I407M	3:4 (3:3)	S6	Reduced current	Mutant exerts dominant negative effect over wild type	Positive shift in voltage dependence of activation	Unpublished data, SE Tomlinson *et al.*
4	R167M	1:1	N-terminus	No current	Mutant exerts dominant negative effect over wild type	Positive shift in voltage dependence of activation	
5	C185W	1:2	S1				
6	T226R	1:1 (0:1)	S2	Reduced current	Mutant exerts dominant negative effect over wild type	No change in kinetics when co-expressed with wild type	Zuberi *et al.*, 1999; Kinali *et al.*, 2004
7	A242P	1:3 (0:3)	S2	Current reduced to 10% of wild type	Loss of function only		Eunson *et al.*, 2000
8	F184C	3:2 (1:1)	S1	Reduced current	Unknown	Positive shift in voltage dependence of activation	Van Dyke *et al.*, 1975; Adelman *et al.*, 1995
9	G311S	1:1 (1:0)	Intracellular C-terminus of S4	Current approx 25% of wild type.	Unknown	Less steeply voltage dependant; pronounced C-type inactivation	Zerr *et al.*, 1998

a Numbers in parentheses indicate reduced numbers included in the data analysis. F = female; M = male.
b These two families may have been related.

motor axons. From these measurements, the rheobase and the strength–duration time constant could be estimated using Weiss' law (Bostock *et al.*, 1983; Mogyoros *et al.*, 1996).

Response to subthreshold conditioning stimuli

Threshold electrotonus refers to the changes in threshold current that occur during and after a subthreshold conditioning stimulus and reflects the electrotonic changes in membrane potential (Bostock and Baker, 1988). The conditioning stimuli were 100 ms in duration, applied at specified percentages of the unconditioned threshold current (i.e. 20% and 40% of threshold) in both the hyperpolarizing and depolarizing directions. The change in threshold was tested at different time points during and up to 100 ms after the 100 ms conditioning current. An example of measurements made during this part of the test are reported as follows: TEd^{20}(40–60 ms) where TE is measured as the percentage change in stimulus required to produce a constant action potential during and after a subthreshold conditioning current; 'd' or 'h' represents a depolarizing or hyperpolarizing conditioning stimulus, respectively; the strength of conditioning stimulus is indicated in superscript; and the time relative to the start of the conditioning current is indicated in parentheses.

Just as threshold electrotonus is a threshold analogue of electrotonus, the current/threshold relationship is a threshold analogue of the current/voltage (*I*/*V*) relationship, measured in the TrondE protocol at the end of 200 ms conditioning currents. Prolonged hyperpolarizing currents produce inward rectification, whereas depolarizing currents produce outward rectification, with an increase in the slope of the curve (analogous to conductance) in each case. For these measurements, the strength of the 200 ms polarizing current was altered in 10% steps from 50% depolarizing to 100% hyperpolarizing.

Response to supramaximal conditioning stimuli

The recovery cycle was measured using a supramaximal conditioning stimulus and tracking threshold at various time points, up to 200 ms, after the conditioning stimulus. Before measuring the response to the test stimulus, the response to the conditioning stimulus delivered alone was subtracted from that to the conditioning + test stimulus pair. The phases of the recovery cycle comprise the relative refractory, superexcitable and late subexcitable periods.

On completion of the recording, temperature was recorded at the site of nerve stimulation. Definitions of relevant measures of axonal excitability are given in Table 2.

Statistical analysis

Since for many excitability parameters, the values in patients were more variable than those in control subjects, mean values for the two groups were compared by Welch's unequal variance *t*-test (Table 3). Values for relative refractory period and rheobase were normalized by log conversion before averaging. An ANOVA was also carried out to compare the excitability measures within and between mutations. Because some excitability parameters are significantly related to age (Jankelowitz *et al.*, 2007*b*; Bae *et al.*, 2008) and some to skin temperature (Kiernan *et al.*, 2001*a*), all parameter values were corrected to an age of 40 years and a skin temperature of 33.5°C, using the relationships in the control subjects, before the

Table 2 Definitions of nerve excitability measurements used to compare patients with episodic ataxia type 1 and controls (see Table 3)

Measurement	Definition
Recovery cycle	
Relative refractory period	Interstimulus interval at which refractoriness ends and superexcitability begins (Fig. 1Cc)
Superexcitability	Maximal % threshold reduction[a]
Late subexcitability	Maximal % threshold increase after 10 ms[a]
Threshold electrotonus	
TEd^{20}(peak)	Peak % reduction in threshold during depolarizing currents set to 20% of the resting threshold[b]
TEd^{40}(peak)	Peak % reduction in threshold during depolarizing currents set to 40% of the resting threshold[b]
TEd^{40}(90–100 ms)	Mean percentage threshold reductions between the specified latencies for the 40% depolarizing current
TEd^{40}(undershoot)	Minimum percentage threshold reduction after the 100 ms depolarizing current[b]
TEd^{40}(accommodation)	Maximum drop from TEd^{40}(peak) during 100 ms depolarization[b]
TEh^{40}(90–100 ms)	As TEd^{40}(90–100 ms) but hyperpolarizing
TEh^{20}(90–100 ms)	As TEh^{40}(90–100 ms) but during 20% hyperpolarizing current
TEh^{40}(overshoot)	Maximum percentage threshold reduction after the 100 ms hyperpolarization[b]
Current–threshold relationship	
Resting current/voltage slope	The slope of the current-threshold relationship in Fig. 3A, calculated from the polarizing currents −10% and +10% of threshold (see Fig. 3Ba)
Minimal current/voltage slope	Minimum of the curve in Fig. 3B
Hyperpolarizing current/voltage slope	The leftmost point in Fig. 3B
Strength–duration relationship	
Strength–duration time constant	Estimated from the negative intercept on the *x*-axis of the plot of stimulus charge versus stimulus duration (see Fig. 3Cb)
Rheobase	Estimated from the slope the charge-duration plot (Fig. 3C)

a Measurements averaged over three adjacent points.
b Measurements averaged over 20 ms. See also the glossary for definition of terms.

statistical tests were applied. (The values of 40 years and 33.5°C were chosen as lying between the mean values in controls and patients, i.e. 39.1–44.3 years and 33.25–33.58°C, respectively). In Fig. 4, sensitivity was calculated as TP/(TP + FN) and specificity as TN/(TN + FP), where T = true, F = false, N = negatives and P = positives; the cut-off levels were selected to maximize the sum of sensitivity and specificity. The data analysis and plotting were carried out with the Qtrac software, for which some new functions were added by H.B.

Results

Clinical and genetic information

Twenty-nine patients with a history of episodic ataxia were recruited from 10 families in four countries. All patients carried mutations in the *KCNA1* gene (Table 1). Two families carried the same mutation, and may be distantly related (Graves *et al.*, 2010). The remaining eight families carried different mutations. Nine patients were excluded from the final analysis. Two patients from Family 1 were pregnant at the time of the research visit and nerve excitability studies were not undertaken (even though no adverse effects of nerve excitability testing during pregnancy were expected). One subject, a 40-year-old female, was excluded because she also carried a muscle sodium channel *SCN4A* mutation with a paramyotonia phenotype (Rajakulendran *et al.*, 2009), which might conceivably have affected the recordings. (However, we note that this subject's recordings showed similar abnormalities to those of the other patients with episodic ataxia type 1, which is not surprising as *SCN4A* is not expressed at the node of Ranvier.) Two elderly patients, aged 85 and 92 years, were >20 years older than the remaining patients and controls and were excluded from the analysis as outliers, although their excitability properties did not differ significantly from the others. Lastly, a further four patients were excluded from analysis because excessive spontaneous activity prevented satisfactory recording from the muscle. The remaining 20 patients (12 female; 8 male) were aged from 19 to 64 years (mean 44.3 years) and 19/20 had clinical or EMG evidence of neuromyotonia/myokymia. They came from nine families harbouring eight different mutations. Their recordings were compared with 30 normal healthy volunteers (14 female; 16 male), aged 21–64 years (mean 39.1 years). Control data included recordings made from subjects in the UK, USA and Australia, thus giving population-matched controls. Mean surface temperature measured over the median nerve at the wrist in patients was 33.58°C (range 32.0–34.9°C) and in the control group was 33.25°C (range 31.0–34.9°C). The studies were well tolerated by all patients.

Nerve excitability studies

The group data for patients and controls are compared in Table 3. The most conspicuous abnormalities in the patients with episodic ataxia type 1 were in the recovery cycle, threshold electrotonus and current–threshold relationship (Figs 1–3).

Table 3 Comparison of nerve excitability parameters between patients with episodic ataxia type 1 and normal control subjects

Excitability parameter	Normal controls (mean ± SE, *n* = 30)	EA1 patients (mean ± SE, *n*)	*t* (df)[a]	*P*
Recovery cycle				
Superexcitability (%)	−22.4 ± 0.8	−44.7 ± 2.2 (20)	10.3 (25.5)	7.1×10^{-10}*****
Subexcitability (%)	14.4 ± 0.7	24.1 ± 1.6 (20)	5.45 (25.1)	1.8×10^{-5}****
Relative refractory period (ms)	2.94× / ÷1.02	2.51× / ÷ 1.02 (20)	5.51 (44.3)	3.9×10^{-6}*****
Threshold electrotonus				
TEd^{40}(peak) (%)	67.9 ± 0.7	74.9 ± 0.6 (18)	7.58 (44.3)	9.5×10^{-9}*****
TEd^{40}(90–100 ms) (%)	43.9 ± 0.7	46.0 ± 1.3 (18)	1.45 (26.1)	0.15^{NS}
TEd^{40}(accom) (%)	23.9 ± 0.5	35.8 ± 1.5 (18)	7.70 (21.0)	3.2×10^{-7}*****
TEd^{40}(undershoot) (%)	−18.5 ± 0.6	−25.8 ± 1.2 (18)	5.54 (24.6)	1.5×10^{-5}****
TEd^{20}(peak) (%)	38.0 ± 0.5	49.9 ± 1.5 (20)	7.43 (23.3)	3.0×10^{-7}*****
TEh^{20}(90–100 ms) (%)	−46.9 ± 1.0	−64.3 ± 2.3 (20)	6.91 (26.2)	5.3×10^{-7}*****
TEh^{40}(90–100 ms) (%)	−116.4 ± 2.8	−146.5 ± 4.9 (18)	5.33 (27.6)	1.8×10^{-5}****
TEh^{40}(overshoot) (%)	13.8 ± 0.6	21.1 ± 1.7 (18)	4.12 (20.9)	0.00055***
Current–threshold				
Resting current/voltage slope	0.605 ± 0.014	0.483 ± 0.015 (20)	6.04 (44.5)	8.2×10^{-7}*****
Minimum current/voltage slope	0.246 ± 0.008	0.237 ± 0.012 (20)	0.59 (34.2)	0.57^{NS}
Hyperpolarized current/voltage slope	0.339 ± 0.010	0.390 ± 0.028 (20)	1.71 (24.4)	0.10^{NS}
Strength–duration				
Strength–duration time constant (ms)	0.472 ± 0.017	0.463 ± 0.022 (20)	0.32 (39.8)	0.75^{NS}
Rheobase (mA)	2.80 × / ÷1.04	4.01 × / ÷1.07 (20)	4.81 (32.1)	5.1×10^{-5}****
Subjects/recording[b]				
Age (years)	39.1 ± 2.4	44.3 ± 3.1 (20)	1.33 (39.5)	0.19^{NS}
Sex (M = 1, F = 2)	1.47 ± 0.09	1.60 ± 0.10 (20)	0.92 (41.2)	0.37^{NS}
Temperature (°C)	33.25 ± 0.17	33.58 ± 0.19 (20)	1.27 (43.0)	0.21^{NS}

For definition of excitability parameters see corresponding position in Table 2.$^{NS}P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; *****$P < 0.00001$.
a *t* and degrees of freedom for Welch's unequal variance *t*-test.
b These parameters not corrected for age and temperature. F = female; M = male.

Recovery cycle

The excitability changes following supramaximal stimulation are plotted for all control and patient recordings in Fig. 1A and B, respectively, and the group means and standard errors are illustrated in Fig. 1C. The patient recordings are characterized by 100% greater superexcitability (Fig. 1Ca), 67% greater late subexcitability (Fig. 1Cb) and a 15% shorter relative refractory period (Fig. 1Cc).

Threshold electrotonus

The excitability changes during and after subthreshold conditioning currents set to ± 40% and ± 20% of the unconditioned threshold current are plotted in Fig. 2A, and the responses to the 40% and 20% depolarizing currents are replotted for clarity in Fig. 2B and C. There were greater threshold changes with both depolarizing and hyperpolarizing currents in the patients, producing the so-called 'fanning out' appearance of the threshold electrotonus waveform.

The true extent of the excitability change with the 40% depolarizing current cannot be appreciated because some motor axons discharged at the start of the conditioning current. This results in an exaggerated dip in threshold at 40–60 ms into the depolarizing threshold electrotonus (Fig. 2B), due to activation of slow potassium currents by the action potentials (Trevillion *et al.*, 2007). These features did not occur with the 20% polarizing current and greater emphasis is therefore placed on the 20% data (Fig. 2Cc). The most relevant abnormal features in the patients with episodic ataxia type 1 were a 31% greater peak threshold reduction in response to the 20% depolarizing current (Fig. 2Ca), a 37% greater increase in threshold in response to the 20% hyperpolarizing current (Fig. 2Ab) and a 50% greater accommodation to the 40% depolarizing current (Fig. 2Bc).

Current–threshold relationship

The current–threshold relationship (defined as threshold analogue of current–voltage (*I*/*V*) relationship, measured at end of subthreshold polarizing current) in Fig. 3A, shows the changes in threshold at the end of 200 ms currents ranging from 50% of threshold (depolarizing; top) to −100% of threshold (hyperpolarizing; bottom). The slope of this curve is a threshold analogue of input conductance, which increases towards the top, due to outward rectification associated with the activation of fast and slow K^+ channels, and towards the bottom due to activation of hyperpolarization-activated cyclic-nucleotide-gated channels. The slopes are plotted in Fig. 3B and the most prominent abnormality in the patients was a 20% reduction in the resting slope

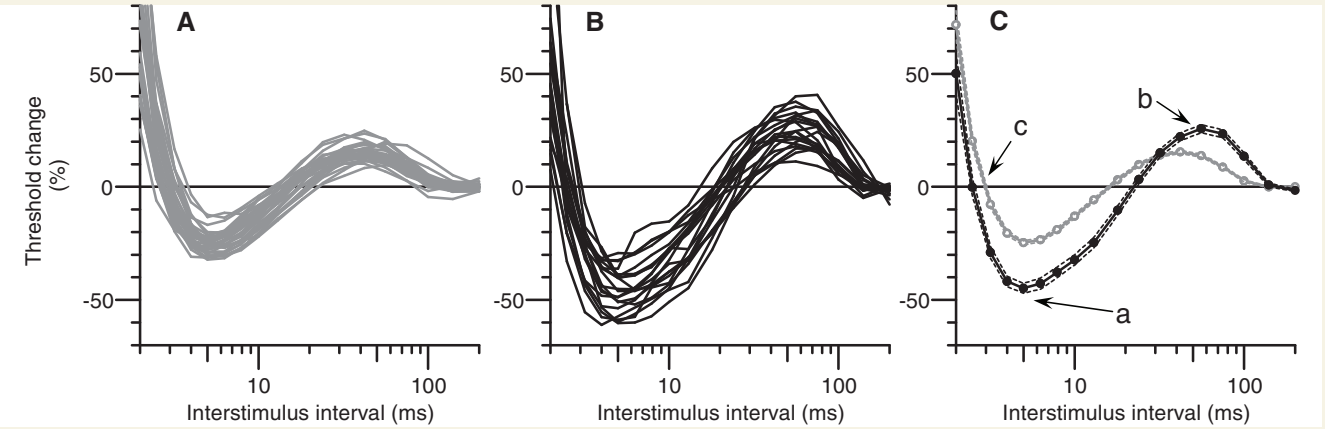

Figure 1 Abnormal nerve recovery cycles in episodic ataxia type 1. Threshold changes are percentage increase in threshold current at different times after a supramaximal conditioning stimulus. (**A**) Superimposed recovery cycles from 30 normal control subjects. (**B**) Recovery cycles from 20 patients with episodic ataxia type 1. (**C**) Mean and standard errors of responses in (**A** and **B**) superimposed: open grey circles = controls; filled black circles = patients. Small letters indicate parameters referred to in text: a = peak superexcitability; b = peak late subexcitability; c = relative refractory period.

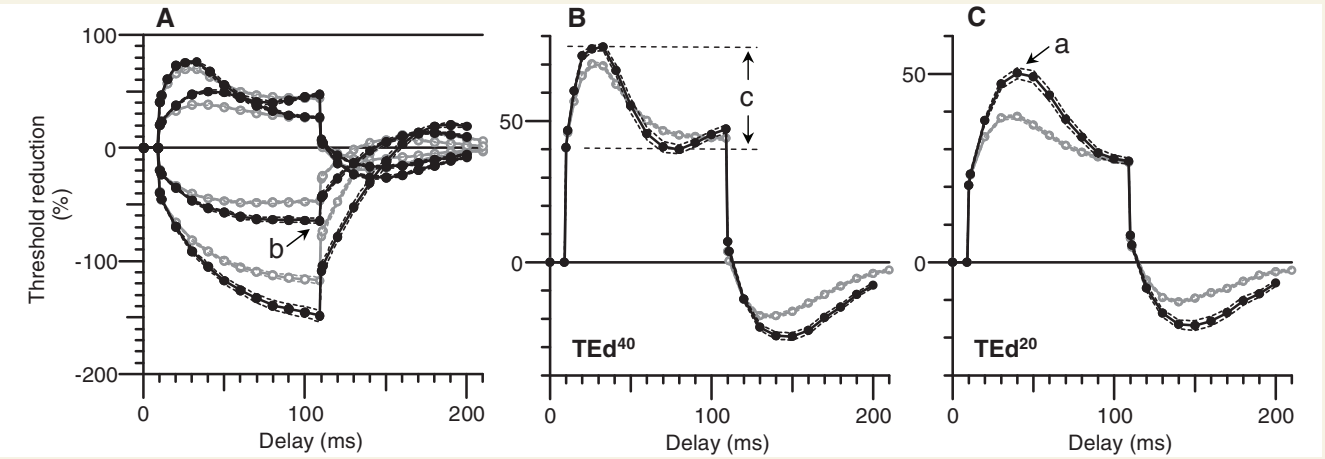

Figure 2 Abnormal threshold electrotonus in episodic ataxia type 1. (**A**) Changes in threshold during and after 100 ms polarizing currents set to +40% and +20%, (depolarizing, top traces), −20% and −40% (hyperpolarizing, bottom traces) of unconditioned threshold current, with decreases in threshold in response to depolarizing current plotted upwards and an increase in response to hyperpolarizing current downwards. Mean and standard errors of controls (grey) and patients with episodic ataxia type 1 (black) plotted as in Fig. 1C. (**B**) Responses to +40% depolarizing currents expanded. (**C**) Responses to +20% depolarizing currents. Letters indicate most significantly altered excitability parameters: a = TEd^{20}(peak), i.e. peak threshold reduction during depolarizing current set to 20% of threshold; b = TEh (90–100 ms); c = TEd^{40}(accom), i.e. maximum accommodative increase in threshold after peak, during 40% depolarizing current.

($P < 10^{-6}$; Fig. 3Ba). This suggests that fewer channels were open at the resting potential.

Strength–duration relationship

The strength–duration data are plotted in Fig. 3C as a charge–duration plot, where 'stimulus-charge' reflects the energy in the stimulus pulse (i.e. stimulus charge = stimulus current × stimulus duration). Strength–duration time constant is given by the negative intercept of the regression line on the *x*-axis, and the rheobase is given by the slope of the regression line. The strength–duration time constants were not significantly different, but the rheobase (i.e. threshold current for very long stimulus pulses) was 43% higher in the patients.

Separation of patients from normal controls by nerve excitability measurements

Figure 4A shows that, in this dataset, all patients had superexcitability in excess of −29.4%, while all controls had superexcitability less than that. Figure 2Ca suggests that the most discriminating threshold electrotonus parameter is TEd^{20}(peak) and with it a

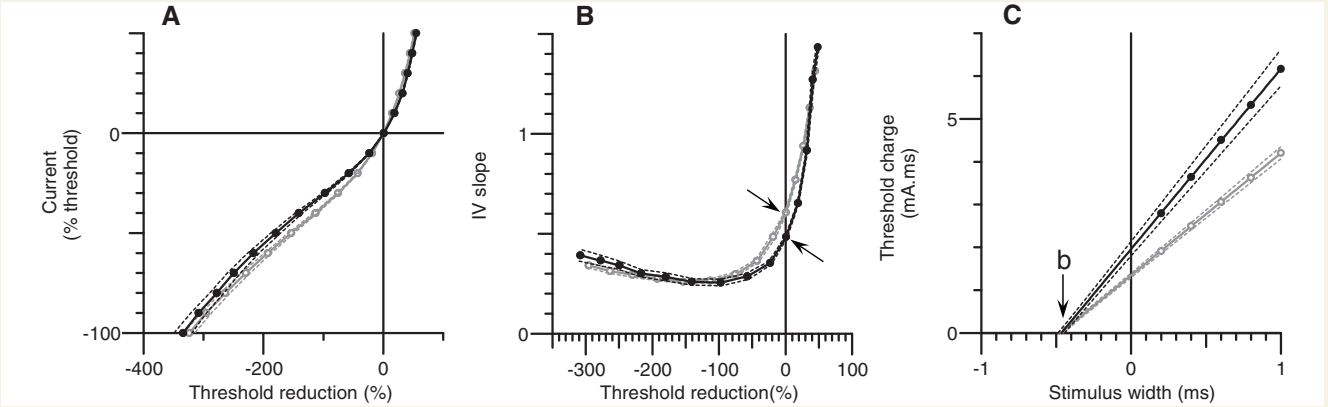

Figure 3 Current–threshold (*I /V*) relationships. (**A**) Threshold changes 200 ms after the onset of long polarizing currents (*x*-axis), plotted against polarizing current (*y*-axis). Filled black circles are means for patients with episodic ataxia type 1 and open grey circles are means of control subjects, plotted with standard errors as in Fig. 1C. (**B**) Slopes of curves in (**A**), showing reduction around the resting potential (arrows indicate zero on the *x*-axis), indicating reduced membrane conductance. (**C**) Weiss Plot of threshold charge (i.e. threshold current × stimulus duration) against stimulus duration in which the negative intercept on the *x*-axis (b) represents the strength–duration time constant.

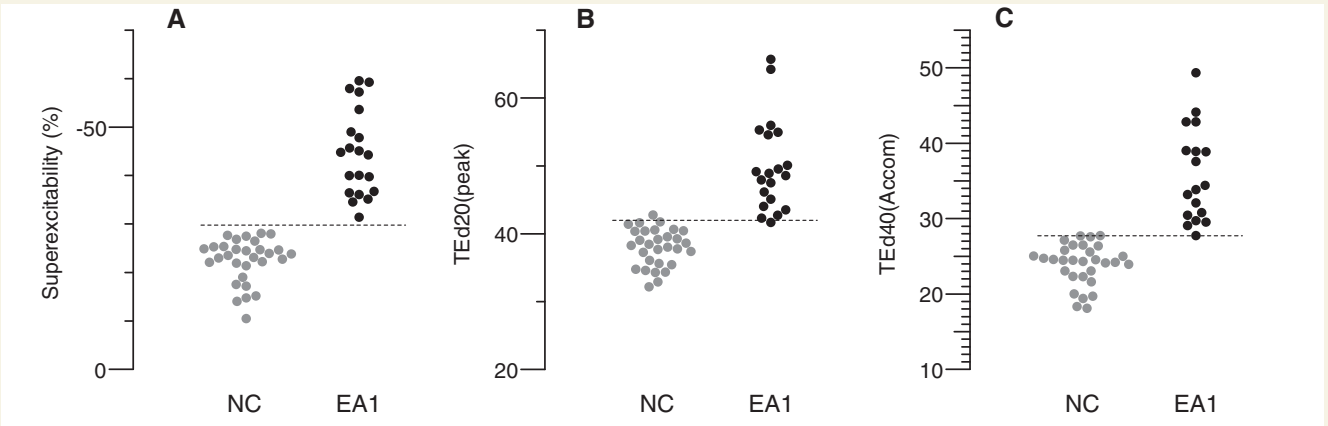

Figure 4 Separation of patients with episodic ataxia type 1 (EA1) from controls (NC) by nerve excitability parameters. (**A**) Superexcitability; (**B**) TEd^{20}(peak); (**C**) TEd^{40}(accom). Dashed horizontal lines are cut-off levels that optimize sensitivity + specificity (see text). Note: data only available for 18 patients in (**C**).

cut-off level of 43.3% separated the patients from controls with a sensitivity of 85% and a specificity of 96.7% (Fig. 4B). For accommodation to 40% polarizing currents (Fig. 2Bb), a cut-off level of 26.4% provided a sensitivity of 100% and a specificity of 90%. In these figures, as in Table 3, the excitability parameters were corrected to allow for their known dependence on age (Jankelowitz *et al.*, 2007*b*; Bae *et al.*, 2008) and temperature (Kiernan *et al.*, 2001*a*; see 'Methods' section). Even without the age and temperature compensation, the figures for optimum sensitivity and specificity were still very high: superexcitability 95% and 100%; TEd^{20}(peak) 85% and 93.3%; TEd^{40}(accom) 94.4% and 100%.

Even clearer separation can be achieved by combining excitability parameters. For example, combining superexcitability and TEd^{20}(peak) in a scatter plot (Fig. 5) shows that not only was there no overlap, but also all the patient observations fell outside the 95% confidence limits for the normal controls.

Different mutations

Figure 5 shows that although patients with episodic ataxia type 1 and controls could be distinguished by superexcitability and electrotonus, the variability of these parameters was considerably greater in the patient group. The *in vitro* expression data in Table 1 indicate that most mutations reduced fast K^+ currents but did not abolish them. To determine if the nerve excitability properties in the patients varied with the nature of the mutation, the values for superexcitability and TEd^{20}(peak) in the patients

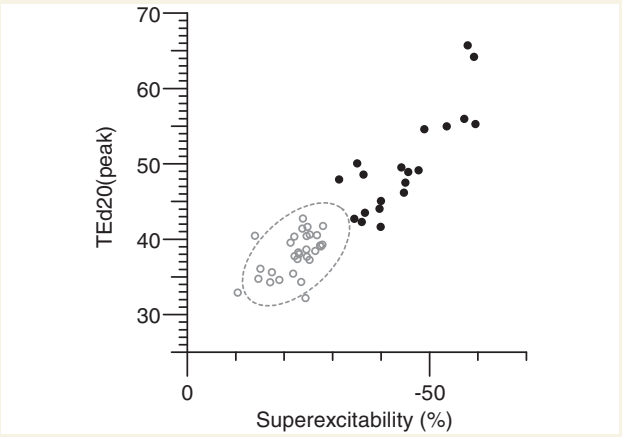

Figure 5 Separation of patients with episodic ataxia type 1 from controls by two nerve excitability parameters. Open grey circles represent individual values and the dashed ellipse represents the 95% confidence limits for combinations of superexcitability and TEd^{20}(peak) electrotonus values for normal control subjects. Filled circles = patients with episodic ataxia type 1. There is a strong correlation between these two excitability parameters, both of which are highly sensitive to $K_v1.1$ dysfunction, and all patients with episodic ataxia type 1 were outside the normal limits.

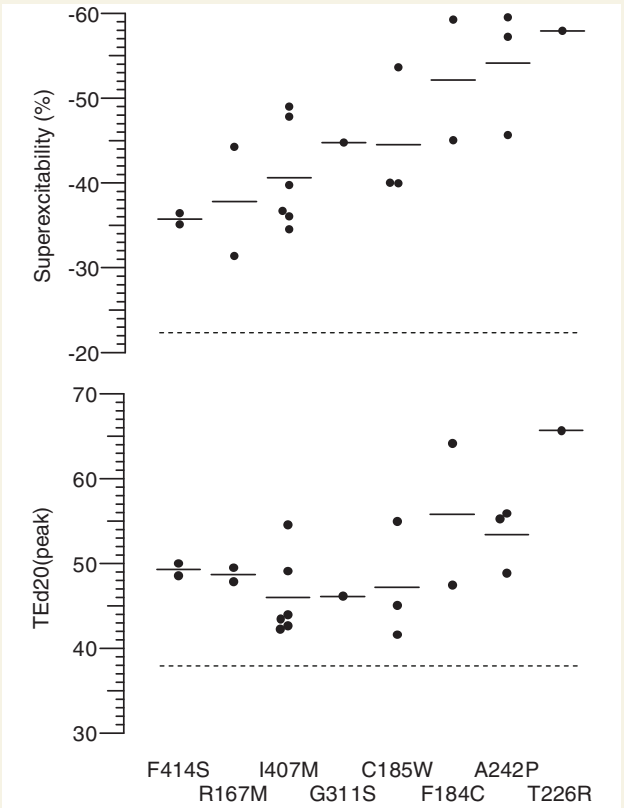

Figure 6 Comparison between different *KCNA1* mutations. Values of superexcitability (upper panel) and TEd^{20}(peak) (lower panel), for patients with episodic ataxia type 1 grouped by *KCNA1* mutation, according to mean superexcitability. Horizontal dashed lines indicate mean values for normal controls.

with episodic ataxia type 1 were plotted for each mutation (Fig. 6). Analyses of variance, comparing variation within mutations with variation between mutations revealed no significant differences for superexcitability or TEd^{20}(peak) ($P = 0.072$ and $P = 0.086$, respectively). The present data thus do not provide evidence that the mutations differ in their effects on these parameters.

Comparison between nerve excitability changes in episodic ataxia type 1 and those in other conditions

The potential utility of excitability measurements as a clinical tool depends not only on the extent of any abnormalities, but also their specificity. Previous findings show that the recovery cycle alone is able to distinguish between several different pathologies (Kiernan *et al.*, 2002*a*,*b*). Figure 7 extends this analysis by showing mean values of superexcitability and late subexcitability from a number of earlier studies, compared with the 95% confidence limits for normal controls. The changes in superexcitability and subexcitability in episodic ataxia type 1 are quite distinct, in the opposite direction to those in chronic renal failure (attributed to increased serum K^+ levels; Kiernan *et al.*, 2002*b*), to Na^+ channel blockade due to tetrodotoxin poisoning (Kiernan *et al.*, 2005*a*) and from all the other groups. There is some overlap, however, between the episodic ataxia type 1 data and those from patients with multifocal motor neuropathy (Kiernan *et al.*, 2002*a*), but it is unlikely that these conditions would be confused clinically.

Discussion

This study has demonstrated that patients with episodic ataxia harbouring mutations in the *KCNA1* gene, encoding the fast potassium channel $K_v1.1$, have distinctive abnormalities in nerve excitability, consistent with expression of this channel in peripheral nerves. Furthermore, 19/20 patients had clinical evidence of axonal hyperexcitability, manifesting as either neuromyotonia or myokymia. However, the clear separation of these patients from controls by excitability measures alone, as seen in Fig. 5, is significant and indicates the potential utility of this technique in the clinical setting. It is of interest to consider why this channelopathy should have these particular effects on excitability, why the excitability changes differ from those reported in Isaac's syndrome (an acquired channelopathy affecting the same channels) and also to consider the potential value of nerve excitability testing as an aid to clinical diagnosis in neuronal channelopathies.

The nature of the nerve excitability changes in episodic ataxia type 1

The nerve excitability abnormalities seen in the patients with episodic ataxia type 1 correspond well to the effects of fast K^+

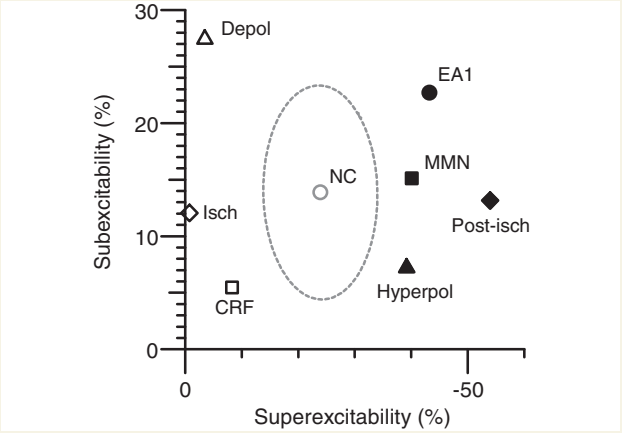

Figure 7 Recovery cycle parameters in episodic ataxia type 1 and other nerve pathologies. The cross and ellipse indicate mean and 95% confidence limits of 30 normal control subjects (data from this study). Other symbols indicate mean values for different conditions. Open and filled triangles = four normal nerves depolarized (Depol) and hyperpolarized (Hyperpol) by 1 mA direct current (Kiernan and Bostock, 2001); open and filled diamonds = four normal nerves after 5-min ischaemia (Isch) for 5 min after release of ischaemia (Post-isch) (Kiernan and Bostock, 2001); filled square = six patients with multifocal motor neuropathy (MMN) (Kiernan *et al.*, 2002*a*); open square = nine patients with chronic renal failure (CRF), before dialysis (Kiernan *et al.*, 2002*b*); open circle = four patients with tetrodotoxin intoxication from puffer fish ingestion (NC) (Kiernan *et al.*, 2005); filled circle = 20 patients in the present study.

channel blockers on nerve action potentials and electrotonus in previous studies on animal preparations. Baker and colleagues (1987) contrasted the effects of 4-aminopyridine (which blocks fast K^+ channels) and tetraethylammonium ions (which block slow K^+ channels) on action potentials, afterpotentials and electrotonus in rat spinal roots. 4-Aminopyridine caused a small depolarization of the resting potential of motor fibres and increased the early electrotonic response to depolarizing currents, corresponding to the increase in early threshold electrotonus. It increased and prolonged the depolarizing afterpotential that gives rise to the superexcitable period, and also increased the late hyperpolarizing afterpotential, which gives rise to late subexcitability. As in episodic ataxia type 1, these effects of reduced fast K^+ conductance can be explained by two factors, the removal of the shunting by one K^+ channel of the effects of the other, and the membrane depolarization that brings slow K^+ channels to a steeper part of their activation curve. The reduction of the contribution of $K_v1.1$ channels to the resting conductance accounts for the alteration of the current–threshold relationship, best appreciated in the plot of current–threshold slope (a measure analogous to conductance, Fig. 3B). The reduced resting conductance of the internodal axolemma also accounts for the 'fanning-out' of threshold electrotonus (Fig. 2Ab). The reduction in resting conductance occurs despite membrane depolarization, which by itself would produce an increase in resting conductance and 'fanning-in' of threshold electrotonus. The reduction in input conductance does not extend to hyperpolarized potentials when the $K_v1.1$ channels are fully switched off (Fig. 2Ab). This was seen in the earlier study of 4-aminopyridine on the current–voltage relationship (see Fig. 4Cc in Baker *et al.*, 1987).

Nerve excitability properties in episodic ataxia type 1 and Isaac's syndrome

Neuromyotonia due to reduced fast K^+ channel function is seen in Isaac's syndrome as well as episodic ataxia type 1. In the former, channel function is reduced by autoantibodies directed against the channels rather than a genetic abnormality (Hart *et al.*, 2002). It might, therefore, be expected that nerve excitability studies would reveal similar abnormalities in the autoimmune and genetic K^+ channelopathies. However, in a study of 11 patients with spontaneous motor unit activity due to autoimmune-associated acquired neuromyotonia or cramp-fasciculation syndrome, none exhibited an abnormally high superexcitability or other evidence of $K_v1.1$ dysfunction that could underlie the hyperexcitability (Kiernan *et al.*, 2001*b*). This was unexpected, especially since three of the patients had evidence of auto-antibodies to voltage-gated K^+ channels of the α-dendrotoxin-binding K_v1 family. This discrepancy in nerve excitability, as tested at the wrist, between the autoimmune and genetic K_v1 channelopathies possibly reflects failure of the autoantibodies to gain access through the blood–nerve barrier to the channels that are concentrated in the juxtaparanodal axolemma, under the myelin sheath (Arroyo *et al.*, 1999). The ectopic activity responsible for neuromyotonia probably arises from axon terminals where the blood–brain barrier is deficient, a conclusion for which there is considerable independent evidence (Torbergsen *et al.*, 1996; Deymeer *et al.*, 1998). Nerve excitability testing may, therefore, provide an indication of whether axonal hyperexcitability is uniform along the axon and probably genetic in origin, or focal and more likely to be autoimmune. Given the sensitivity of these studies, a negative result would render a diagnosis of episodic ataxia type 1 unlikely and, in a patient with neuromyotonia, a search for humoral factors should be undertaken.

Nerve excitability testing as a test for neuronal channelopathies

Neuronal ion channel disorders are often characterized by intermittent symptoms such as seizures, attacks of migraine or paroxysmal ataxia and there may also be fixed or progressive deficits. Structural imaging may be helpful only in excluding anatomical causes of the cerebellar dysfunction and seizures, and a sensitive and specific test of nerve function could be useful. This study on an international cohort of patients with episodic ataxia type 1 with eight different mutations of the *KCNA1* gene has shown that, despite the genetic heterogeneity and variable degrees of neuromyotonia, all subjects expressed a consistent abnormality in nerve excitability that reflected the $K_v1.1$ potassium channel dysfunction. In addition, the same abnormalities were seen in three patients excluded because of age or a coexistent mutation involving the

muscle Na^+ channel. The multiple nerve excitability measurements not only distinguish patients from controls with high sensitivity and specificity, but the qualitative information available is effective at distinguishing this abnormality from others (Fig. 7). Although nerve excitability testing is not currently widely available in the clinical setting, we suggest that further refinement of these techniques has the potential to aid diagnosis of other CNS channelopathies where the responsible ion channel is also expressed in peripheral nerve.

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Conflict of interest: H. Bostock receives royalties from University College London for sales of the Qtrac software used in this study.

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